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Same day plantation establishment of the root hemiparasite sandalwood (*Santalum spicatum* (R Br) A DC: Santalaceae) and hosts

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Abstract

Interest and investment in a plantation sandalwood (*Santalum spicatum* (R Br) A DC) industry in southern Western Australia has been steadily growing over the last few years. Current plantation establishment involves planting host seedlings in year one and then direct sowing of untreated seeds of the parasitic sandalwood in year two or three. An innovative establishment technique in which host seedlings of *Acacia acuminata* Benth and partially germinated sandalwood seeds are planted on the same day was compared to the current establishment methods. The study showed that sandalwood and host establishment in one season is achievable and that it was three times more successful than the most widely used and promoted technique at present. Results also indicated that water availability influenced the germination, summer survival and growth of sandalwood. The use of small seedling hosts on well-watered, cleared land results in a higher rate of sandalwood establishment and growth.

Keywords: sandalwood, *Santalum spicatum*, establishment, host, hemiparasite.

Introduction

The heartwood of the hemiparasitic Western Australian sandalwood (*Santalum spicatum*) contains valuable fragrant oils. The sale of 1 814 tonnes of *Santalum spicatum* in the 1999-2000 financial year generated a revenue of $11 642 000 for the State of Western Australia (Anon 2000). Most wood is sourced from natural populations in the Goldfields, and some is salvaged from dead remnants in the Midwest. Sandalwood was once extensively harvested from the medium rainfall "wheatbelt" areas of the State, but this resource has been exhausted due to over exploitation and clearing for agriculture.

There has been growing interest from the farming community and other investors in the development of a plantation *Santalum spicatum* industry in the wheatbelt. Extensive trials have shown that *Santalum* species show improved growth and vigour when cultivated with leguminous hosts (Radomiljac & McComb 1998; Brand *et al.* 2000; Loveys *et al.* 2001). Current recommendations for *Santalum spicatum* plantation establishment (Brand & Jones 2001) involves the planting of host seedlings (usually *Acacia acuminata* as the sole host species) during winter of the first year. In the second or third year, four untreated sandalwood seeds are sown around each *Acacia* host tree during April or May. While this method can be successful in years with favorable conditions, it requires 3300 (up to 15 kg) sandalwood seeds per hectare and there is a lag phase of up to several years between host and sandalwood establishment, which requires two separate operations. The high seeding rate is required because field germination is generally low, less than 20%, even when seed viability is consistently high at 80-90% (Loneragan 1990).

Although the establishment of sandalwood and hosts in one season may have previously been considered desirable, it was not thought practical as host seedlings are generally planted in July and August whereas the optimum time to sow sandalwood seeds has been shown to be during April and May (Loneragan 1990; Brand & Jones 2001). Also, the field survival of pot-raised sandalwood seedlings (either alone or with a pot host) has not been successful when planted in unirrigated low rainfall (< 600 mm) areas (Loneragan 1990). Loneragan (1990) reported that only 2% of sandalwood seedlings pot grown with *Acacia acuminata* then planted in the field survived their first summer. The aim of this research was to determine whether hosts and sandalwood could be established in the same year and at the same time.

Methods

Site descriptions

Sandalwood germination and survival was monitored at three sites. Sites 1 and 2 were 50 m apart and located 10 km south-west of Borden, Western Australia. Site 3 was located 29 km south of Borden. The soil at sites 1 and 2 was duplex with grey sandy topsoil, 0.3-0.8 m deep, underlain with clay. Site 3 was a red deep loamy duplex, with clay at 0.8 m. Prior to 2000 site 1 had been used for grain production (cereals, lupins and canola). Site 2 was in remnant vegetation that had been partially cleared (> 20 years ago) and had been grazed until 1997. Recruitment of *Acacia acuminata* and *Allocasuarina huegeliana* (Miq) LAS Johnson had occurred at this site after the removal of stock. Site 3 was also used for grain production until 1998, when rows of *Acacia acuminata* were direct seeded (4 m apart) and the site fenced to exclude stock.

Sandalwood establishment

Remnant trees growing within a 20 km radius of Wagin, Western Australia provided the 120 sandalwood seeds that were sown at site 1. Seeds sown at the other two sites were collected from remnant trees growing within a 20 km radius of Borden. The epicarp was removed from all seeds prior to treatment. Glasshouse testing showed that Borden and Wagin seed had similar viability (95%). Seedling emergence of untreated Borden and Wagin seed, sown at other sites in 2000, were not significantly different (data not shown).

All seeds sown at site 1 were cracked using a wetting and drying procedure where seeds were moistened for 6 hours then placed on activated desiccant (silica gel) for 6 hours. This procedure was repeated three times. Cracked seeds were vacuum infiltrated (see Loveys & Jusaitis 1994) with a gibberellic acid solution (500 mM GA_3: Sigma Chemical Co, USA). The solution also contained two fungicides, Fungarid™ and copper oxychloride, used at concentrations recommended by the manufacturers. Seeds were sown (late June 2000) in trays containing a mixture of coarse sand, perlite and peat (2:1:0.5). Trays were watered sparingly and after twenty days when the cracked endocarp had opened 2-3 mm (see Fig 1D,E), they were removed and sown at site 1. Given ideal conditions, the radicle emerged 5-10 days later through the cracked endocarp from the swollen bud (Fig 1E). Site 1 was scalped, ripped and mounded (rows 4 m apart) and sown with a host seed mix (predominantly *Acacia* species at 500 g ha^{-1}) in a one pass operation using a Chatfields tree planter. Immediately, a small *A. acuminata* (< 150 mm tall) seedling was planted by hand on the mounds every 5 m together with a partially germinated sandalwood seed. The top of the seedling roots and the sandalwood seed were both planted 5 cm below the soil surface, and as close to each other as practically possible.

At site 2, untreated seeds (1000) were sown in May 2000 at a depth of 5 cm in rip lines adjacent to regenerating hosts in the remnant bush. At site 3, untreated seeds (120) were sown at a depth of 5 cm during May 2000, as recommended by Brand & Jones (2001).

Emergence, summer survival and growth

Emerged sandalwood seedlings were counted at each site in October 2000. In April 2001, sandalwood seedlings that had survived summer were counted. At site 1, girth measurement at the soil surface was used to assess host and sandalwood growth in May 2001, approximately 10 months after planting the partially germinated sandalwood seeds and *Acacia acuminata* seedlings. Sandalwood girth was also measured at 150 mm above the soil surface, as this is the industry standard (Loneragan 1990).

Plant water status

To determine level of moisture stress at site 1, a pressure chamber (Soil Moisture Equipment Corporation, Santa Barbara, USA) was used to measure midday water status of small branches of *Acacia acuminata* and *Santalum spicatum* in May 2001 (prior to any significant autumn rainfall) as described by Turner (1998).

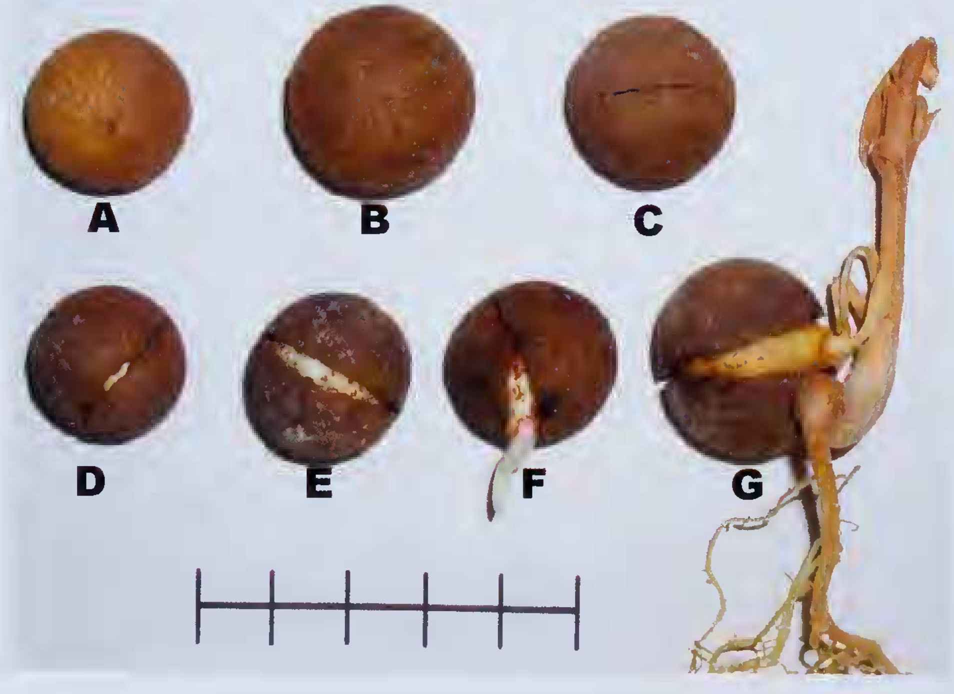

Figure 1. Sandalwood germination sequence. **A**: Dry seed with fine crack at seed apex. **B**: Moistened seed, note fine crack is no longer visible. **C**: Opening of crack after sowing. **D**: Swollen kernel visible as endocarp crack widens. **E**: Kernel between the crack is swollen and raised forming a bud from which, the radicle emerges 5-10 days later given ideal conditions. **F**: Germinated seed with emerged radicle. **G**: Seedling with developing shoot and root system. Seeds similar to those shown in **D** and **E**, were sown at site 1. Scale bar increments are 10 mm, 50 mm total. Note that the time taken to reach each developmental stage varies according to the growing conditions.

Statistical analyses

Genstat™ 5 release 4.1 program (Rothamsted, UK) was used for all statistical analyses.

Rainfall data

Rainfall data for Borden were obtained from the Western Australian Bureau of Meteorology, Perth.

Results

Rainfall

Rainfall at Borden for the year 2000 was 86 mm below the long term average of 381 mm. Rainfall received during the crop growing season (April-October) was 113 mm below the long term average. Drought conditions persisted during the summer and autumn of 2000-2001 (Fig 2).

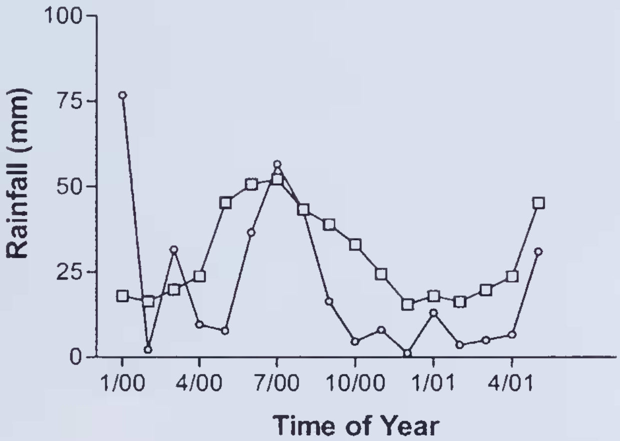

Figure 2. Total monthly rainfall at Borden during 2000-2001 (o) compared to long term averages (□).

Emergence and summer survival

Seventy per cent of all partially germinated sandalwood seeds sown at site 1 produced a seedling that emerged from the soil surface (Fig 3A). This percentage emergence was approximately three times higher than that observed for untreated seeds at the other sites (Fig 3A). At numerous other locations, in the southern Western Australia, field emergence of untreated seeds was generally less than 25% (J Brand, Department of Conservation and Land Management, personal communication). At site 1, 90 % of seedlings survived their first summer (Fig 3B), thus over 60 % percent of the partially germinated seeds produced a seedling that was alive at the end of the summer period (Fig 3C). Similar summer survival was observed at the other sites, consequently only 15-20 % of seeds sown produced a seedling that was alive at the end of the summer period (Fig 3C). These results show that establishing sandalwood and hosts in a "same day" operation is achievable and is more successful, under the dry conditions experienced during 2000-2001, than the technique currently used to establish sandalwood (*ie.* hosts established one to several years prior to sandalwood establishment *e.g.* Site 3).

Growth and water relations

Sandalwood growth at site 1 (10 months after sown in the field) was related to the growth of the *Acacia acuminata* host (adjusted $r^2 = 0.62$; $P < 0.001$; Fig 4A). The biomass of host and sandalwood at this time appeared similar (Fig 5). Sandalwood growth during the first year in remnant vegetation (site 2) was significantly less (sandalwood mean diameter at the soil surface of 2.5 mm, least significant difference (LSD) 1.7 mm) than that measured at the other sites, 7.8 (site 1) and 7.1 mm (site 3). Mean diameter at 150 mm above the soil surface (industry standard) at sites 1 and 3 was 5.2 and 4.2 mm respectively. Most sandalwood seedlings at site 2 were less than 150 mm tall.

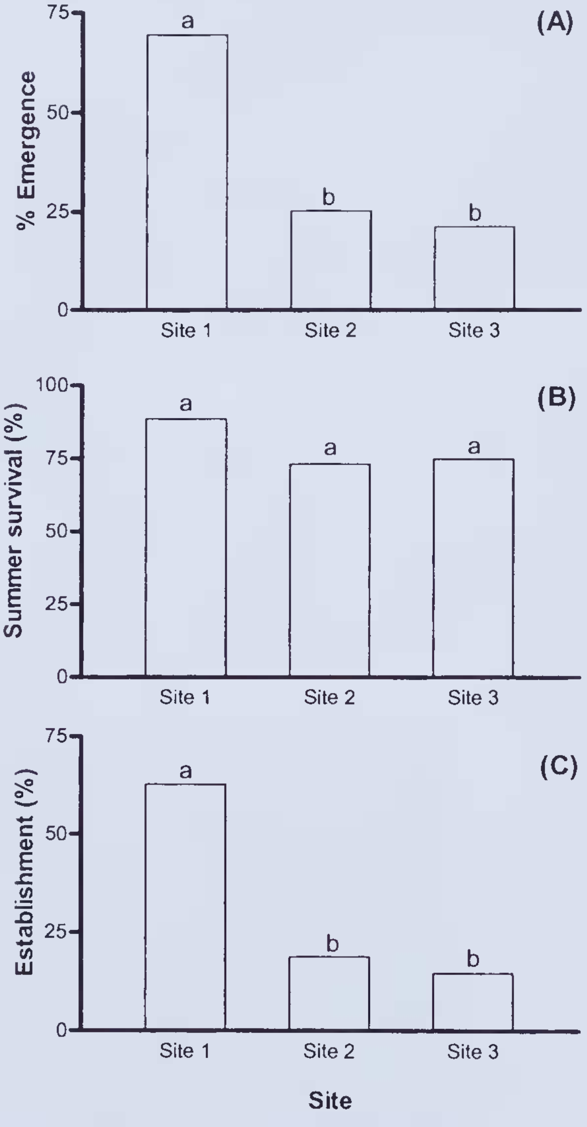

Figure 3. A: Percentage of sandalwood seeds that produced an emergent seedling at the three sites. B: Summer survival of sandalwood seedlings at the three sites (%). C: Percentage establishment at the three sites (ie. percentage of sandalwood seeds that produced an emergent seedling which survived over summer). Within each graph, bars with different letters are significantly different at the 0.05 level of significance (least significant difference determined from a generalised analysis of variance).

The relationship between growth and water status was investigated in May 2001 at site 1. Plant water status influenced the growth of both the sandalwood and the host at this site. Water status accounted for 52 % and 42 % of the observed variation in host and sandalwood growth

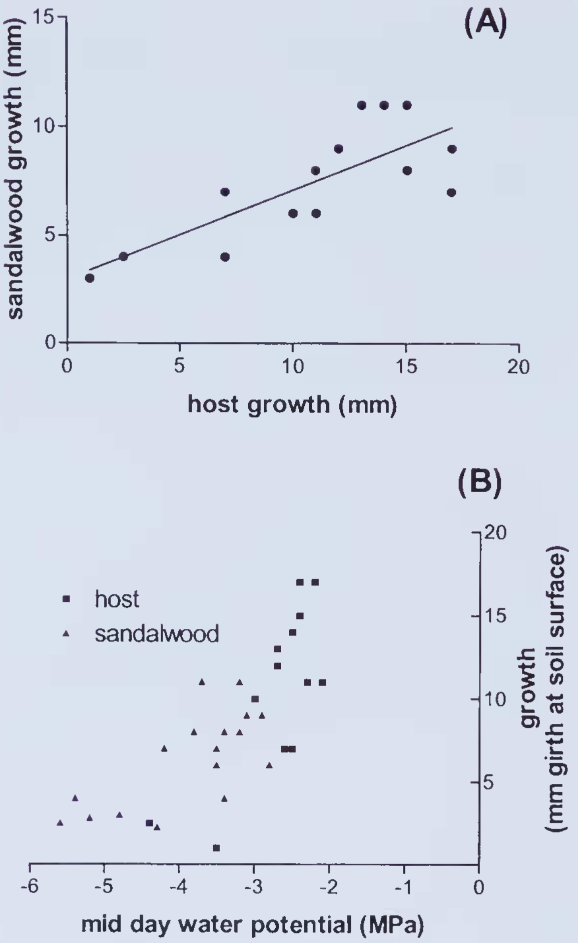

Figure 4. Sandalwood growth as related to (A) host growth (adjusted $r^2 = 0.62$ $P < 0.001$) and sandalwood and host growth as related to (B) plant water status (adjusted $r^2 = 0.54$, $P < 0.001$).

respectively (Fig 4B). Host and sandalwood growth increased with increasing water potential in a linear relationship (adjusted $r^2 = 0.54$, $P < 0.001$; Fig 4B). Mean midday water potential of sandalwood was significantly lower (-3.9 MPa) than that of their adjacent host (-3.2 MPa). It was observed that some sandalwood seedlings which had not survived summer were beside host seedlings (*Acacia acuminata*) that had died; others were beside host seedlings of low water potential (data not shown), suggesting that these sandalwood seedlings may have died from moisture stress.

Discussion

This study has clearly shown that sandalwood and host establishment in the same season is achievable with a 70 % establishment rate and that it was more successful than current techniques under the dry conditions experienced during 2000-2001. Establishment of host and sandalwood in the same year reduces the length of time to harvest by one to two years and would provide cost savings (labour and machinery time) relative to separate introduction of sandalwood in subsequent years. The process of partially germinating seeds would be more expensive than using untreated seeds, but the quantity of partially germinated seeds required per hectare would be approximately a third of that required by the standard technique. A limiting factor to the sandalwood plantation industry may be a seed shortage of local or better provenances in coming years. Brand *et al.* (1999) showed that local provenances are likely to perform best in terms of plantation growth. Sandalwood seed from wheatbelt provenances is limited because most of the original populations have been removed. Therefore, it is important that as little seed as possible is used to establish plantations in the immediate future.

Figure 5. Sandalwood and host growth at site 1. **Left:** rooting morphology of host (H) and sandalwood (SW); note the thickened sandalwood root and haustorial attachment into the main taproot of the host. **Right:** Shoot and root growth of host, planted as a small seedling, and sandalwood, planted as a partially germinated seed; note the other hosts that have germinated in the rip line. Scale bar increments are 0.1 m.

The roots of young sandalwood seedlings are brittle (Herbert 1925; Barrett 1987) and growers who have tried to plant young sandalwood seedlings, either planted alone or with a pot host, have noted that many of the seedlings are damaged even when handled carefully. Haustorial connections that may have formed between the pot host and the sandalwood are also brittle and are easily broken during transplantation into the field. The radicle and plumule of the partially germinated seeds used in this study had not emerged when planted in the field (see Fig 1). These partially germinated seeds were easy to handle and were not damaged during the transplantation process. This avoidance of damage to the germinating sandalwood seed may have led to improved germination and survival.

Replacement of annual crops and pastures with perennial plants results in a drying of the soil profile (Harper *et al.* 2000). The principal hosts of sandalwood are perennial and the current establishment technique recommends that the hosts be planted one or two years prior to the sandalwood (Brand & Jones 2001). The extent to which perennials, in this case acacias, reduce soil moisture would be dependent on their transpiration. The transpiration of a plantation is dependent on the energy balance and leaf area index of the canopy, on the physiological control by the stomata of water loss from the leaves, on the humidity of the air adjacent to the leaves in the canopy, on the availability of water in the soil and on the supply of water by the conducting tissue (Barrett *et al.* 1996). It is apparent therefore that the relatively low sandalwood establishment at sites 2 and 3 during a dry year in the presence of older hosts (> 2 years old) could be due to reduced soil moisture (compared to site 1), and that these lower levels of soil moisture were in part due to the presence of the larger hosts themselves. At site 1, which was cleared land with good weed control, there was adequate moisture available over a sufficient time period, enabling complete germination of the sandalwood seeds and successful attachment to a host root before the soil topsoil dried out during spring through to summer.

Plant water status measured at midday reflects the level of moisture stress experienced at that time (Ritchie & Hinckley 1975). In this study there was considerable variation in the level of water stress experienced by young seedlings at site 1 and this is the first study to show that increasing levels of water stress reduced sandalwood and host growth. In some parts of site 1 the small host seedlings were presumably able to access soil moisture derived from the current years' rainfall and moisture stored in soil profile that had not been removed by previous annual crops and pastures.

Although other potential hosts were direct seeded on the day of sandalwood/*Acacia acuminata* planting, many of these germinated later in 2000 and were not considered capable of supporting the sandalwood over the dry summer-autumn period. Recent excavation (Fig 5) revealed that it was the *Acacia acuminata* seedling that supported the parasite over the dry summer period. It is likely that as the direct seeded hosts develop adjacent to the original *Acacia acuminata* seedling that they too will be eventually parasitised by the sandalwood.

This current study has shown that sandalwood and host can be established on the same day. It was not the intention of the study to determine which parts of the "same day establishment" methodology were most critical, but to demonstrate that "same day establishment" was achievable and to show the benefits relative to other current techniques. From the results we were not able to determine whether the partial germination of the sandalwood nuts was or was not critical for same day sandalwood establishment. However, success was most likely due to the young hosts and attached sandalwood being able to access adequate water in the absence of mature hosts, and the selection and use of sandalwood seeds that have already begun the process of germination. The technique of partially germinating sandalwood seeds in controlled conditions takes only 15-20 days to achieve a state that may take 8 weeks in the field. This process ensures that all seeds sown at least begin germination, which cannot be guaranteed when untreated seeds are sown directly into the field. Once planted into moist soil, the partially germinated seeds will continue to grow and are considered more likely to make haustorial contact with the host planted along side them than an untreated field sown seed that is endeavouring to grow through soil with lower water availability under mature hosts. The value of the process is even greater in dry years like 2000.

Parasitism of hosts by sandalwood can weaken and cause the death of the host, particularly when young (Herbert & Gardner 1921). Brand *et al.* (1999) concluded that differences in host survival between *S. spicatum* planting years might have been related to the initial period of growth without a root parasite. In a subsequent study (Brand *et al.* 2000) the sandalwood was not sown until the *Acacia acuminata* were 5 years of age and over the following three years the sandalwood did not adversely affect host survival. Thus, it was recommended that *Acacia acuminata* seedlings need to be at least 1 m tall before introducing sandalwood (Brand & Jones 2001). The current study has shown that small *Acacia acuminata* hosts (< 1 m tall) can survive and support a young sandalwood seedling of similar size. Indeed sandalwood growth on such small hosts during a very dry year was impressive, with a mean diameter of 5.2 mm at 150 mm above the soil surface at 9 months of age. Similar sandalwood growth rates, 5-8 mm per year, have been observed on larger hosts (Brand *et al.* 1999, 2000). In this study, even if the primary *Acacia acuminata* hosts that were planted as seedlings next to each partially germinated sandalwood seed die in the future, there are numerous other *Acacia* hosts available (including *A. acuminata*) that have germinated and grown as a result of the direct seeding. These additional hosts should provide water and nutrients to the sandalwood and ensure that sandalwood survival and vigor is maintained.

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